应用与环境生物学报 2011, 17 (3): 404~406
Chin J Appl Environ Biol=ISSN 1006-687X
2011-06-25
DOI: 10.3724/SP.J.1145.2011.00404

Sex-linkage Identification of Microsatellite TUT1*

WANG Jie[1, 2], YANG Chen[1], ZHU Lei[2], JIA Chenxi[2] & SUN Yuehua[2**]
([1]*Chengdu Institute of Biology, Chinese Academy of Sciences*, Chengdu 610041, China)
([2]*Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences*, Beijing 100101, China)

Abstract The microsatellite TUT1 was used in several grouse species as an autosomal locus with "heterozygote deficit", but recently found with null alleles. By amplifying in 45 adult Chinese grouse (*Tetrastes sewerzowi*) and five broods with known parents, it was found that TUT1 was homozygous in heterogametic females and heterozygous in 84% males, and transmitted from fathers to both sons and daughters but from mothers to sons only. By BLAST query, a nucleotide sequence (460 bp) that contains TUT1 site was most similar to a portion of the sequence of Z chromosome of chicken (*Gallus gallus*), with the max score of 329 and expect value of 8e-87. Both methods showed that TUT1 was based on Z chromosome. These findings indicated that microsatellite "null alleles" might be originated from sex-linkage and highlighted the importance of gender-specific analysis when publishing and using microsatellites. Tab 1, Ref 17

Keywords microsatellite; null allele; sex-linkage; grouse
CLC Q953.43

微卫星位点TUT1的性别连锁检验*

王 杰[1, 2] 杨 陈[1] 朱 磊[2] 贾陈喜[2] 孙悦华[2**]
([1]中国科学院成都生物研究所 成都 610041)
([2]中国科学院动物研究所动物生态与保护生物学重点实验室 北京 100101)

摘 要 微卫星位点TUT1曾被认为位于松鸡科鸟类的常染色体上，只是多态性低于期望值，然而最近发现存在空基因现象. 通过在45只斑尾榛鸡（*Tetrastes sewerzowi*）亲本和5巢双亲已知的幼鸟上扩增，发现TUT1在异配体的雌性中为纯合体，在84%的雄性中为杂合体，而且可从父本遗传给雌性和雄性后代，但从母本只能遗传给雄性后代. 通过BLAST查询，含TUT1位点的序列（460 bp）与鸡Z染色体的序列最相似，分值329，期望值8e-87. 以上结果表明TUT1位点位于松鸡科鸟类的Z染色体上；提示微卫星位点的空基因现象可能来自性别连锁，因此，在发表和应用微卫星位点时应当进行性别连锁检验. 表1 参17

关键词 微卫星；空基因；性别连锁；松鸡
CLC Q953.43

Microsatellites provide superb tool for population genetics study and conservation/management of biological resources [1]. However, null alleles of microsatellites often occur and can cause egregious errors [2]. Null alleles are mainly due to nucleotide sequence divergence (e.g. point mutations or indels) in one or both primer binding sites that prevent consistent amplification [3]. Another spurious source of null alleles involves sex linkage, wherein in diploid organisms the heterogametic sex carries only one allele at a locus housed on a sex chromosome [2]. Thus, if sex linkage goes unrecognized at a locus, heterogametic sexes will be treated to be homozygous and an associated 'heterozygote deficit' might be misconstrued as indication of null alleles [2]. A sex-linked marker in some circumstances facilitates kinship analyses because of its "brute-force" of parentage exclusion [4] and helps to identify gender in particular taxa [5]. So far, relative few examples of sex-linked microsatellites have been published [2].

Received: 2011-02-17 Accepted: 2011-03-29
*Supported by the National Natural Science Foundation of China (Nos. 31071931, 30620130110)
**Corresponding author (E-mail: sunyh@ioz.ac.cn)

The microsatellite TUT1 was originally cloned in capercaillie (*Tetrao urogallus*) [6] and used as an autosomal locus in several grouse species [7~9]. However, its observed heterozygosity was quite lower than expected heterozygosity [6~9], e.g. 0.60 vs. 0.83 in capercaillie [6], and Larsson *et al*. (2008) found repeated evidence of null alleles for TUT1 [10]. To clarify the potential sex-linkage of a microsatellite and provide some insights on the analysis methods, we performed a pedigree study of TUT1 in Chinese grouse (*Tetrastes sewerzowi*) and aligned the TUT1 sequence with a chicken (*Gallus gallus*) genome [11].

1 Material & Methods

Fourteen females and thirty-one males were captured using walk-in traps from November 2006 to May 2009 at the Lianhuashan Nature Reserve, Gansu, China. Blood (1 mL) was sampled from the brachial vein of all captured adults and marked with necklace transmitters, and colored plastic tarsus bands for individual identification [12]. Thirty-five eggshell-membranes were collected in five clutches with known parents (Table 1).

DNA fragments of TUT1 in all samples were amplified

Table 1 Inheritance and genotypes of five broods of Chinese grouse at the microsatellite TUT1 locus

Individual	B3 nest		B5 nest		A6 nest		S6 nest		S7 nest	
	Sex	TUT1	Sex	TUT1	Sex	TUT1	Sex	TUT1	Sex	TUT1
Mother	F	172	F	180	F	172	F	172	F	172
Father	M	164/180	M	164/172	M	168/180	M	164/172	M	164/172
Chick 1	F	180	M	172/180	F	168	M	172/172	M	164/172
Chick 2	M	180/172		unhatched	F	180	M	164/172	M	164/172
Chick 3	F	164	F	164	M	168/172	F	172	M	164/172
Chick 4	M	164/172	F	172	F	168	M	172/172	M	164/172
Chick 5	M	164/172	F	172	M	180/172	M	164/172	F	172
Chick 6	M	180/172	F	172	M	168/172	M	164/172	F	172
Chick 7	F	164	M	172/180	F	168	F	164	M	172/172
Chick 8	F	164		-		-		-		-

Alleles are identified by size (base pairs). M: male; F: female

[6] and genotyped using an ABI 3730 capillary sequencer. Parentages of the sampled broods were also supported by the analysis on other eight microsatellites [13]. Adults were sexed by the presence of the black chin patch in males [14] and offspring by amplifying the chromo-helicase-DNA-binding (CHD) genes [15]. Allelic sequences of one female and one family (including father, mother, son and daughter) were determined and deposited in GenBank under accession numbers GU462141 and GU738013-8. The sequence similarity of TUT1 in Chinese grouse and capercaillie (AF254653.1) with a chicken genome (AC189016.1) were analyzed using CLUSTAL W in MEGA v3.1 [16]. The polymorphism of TUT1 was determined by CERVUS v3.0 [3] and the deviance from Hardy-Weinberg equilibrium by GENEPOP v4.0 (Fisher's method) [17].

2 Results

All females were homozygous whereas 84% of males were heterozygous at the TUT1 locus. Every son inherited alleles from both parents whereas every daughter carried only one allele from father (Table 1). The TUT1 sequences in capercaillie were most similar to a region of the Z chromosome of chicken, with the max score of 329, query coverage of 71%, max indent of 81%, and expect value of 8e-87, which indicated that TUT1 was based on Z chromosome.

TUT1 was highly polymorphic among male adults (N = 31), with observed alleles of 7, polymorphic information content of 0.748, and observed and expected heterozygosity of 0.796 and 0.839, respectively. It did not deviate from the Hardy-Weinberg expectations, with an F_{IS} of 0.796.

3 Discussion

TUT1 could also be successfully amplified in *Tetrastes bonasia*, *Lagopus lagopus*, *Tympanuchus phasianellus* and *Centrocercus urophasianus* (HÖGLUND Jacob, personal communication), altogether in 5 of 7 genera and 7 of 18 species in Tetraoninae. We failed to amplify TUT1 in blood pheasant (*Ithaginis cruentus*) and chicken, possibly because the binding sites do not fit, thus TUT1 may be specific for Tetraoninae. Considering that TUT1 is hypervariable and not deviated from Hardy-Weinberg equilibrium that has been found, and can be precisely determined (four bytes repeats), it is broadly suitable for the kinship and demography analysis for grouse.

Sex linkage remains a noneliminated source of potential error in most literature reports of 'null alleles' [2]. If sex linkage goes unrecognized at a locus and heterogametic sexes are treated to be homozygous, the associated 'lower observed heterozygosity than expected' might be used as evidence of genetic impoverishment due to the inbreeding in some isolated or threatened populations [7~9]. A 'homozygous parent' might be falsely excluded for an offspring displaying a different 'homozygous' phenotype, if in fact both actually have only one allele that the parent has not inherited to the offspring, like TUT1 from mothers to daughters (Table 1). A locus might also be unfortunately discarded because of the repeated null alleles in heterogametic sexes [10]. Suitable microsatellites are not easy to identify in all species, and careful gender-specific analyses combined with inheritance study can identify the potential sex linkage of microsatellites and thus avoid wrong use. These findings highlight the importance of gender-specific analyses when publishing and using microsatellites. Complete genome maps of many heterogametic organisms have been published (http://www.ncbi.nlm.nih.gov/mapview/), sequence similarity analysis of microsatellites with published genomes may shed insights expediently on their position (i.e. at which chromosome).

Acknowledgments We are particularly grateful to HÖGLUND Jacob, SEGELBACHER Gernot, HAN Zhiming, SONG Gang and YANG Xiaonong for their comments on the manuscript.

References

1 Jarne P, Lagoda PJL. Microsatellites, from molecules to populations and back. *Trends Ecol Evol*, 1996, **11**: 424~429

2 Dakin EE, Avise JC. Microsatellite null alleles in parentage analysis. *Heredity*, 2004, **93**: 504~509

3 Marshall TC, Slate J, Kruuk LEB, Pemberton JM. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol*, 1998, **7**: 639~655

4 Walker D, Power AJ, Avise JC. Sex-linked markers facilitate genetic parentage analyses in knobbed whelk broods. *J Hered*, 2005, **96**: 108~113

5 Avise JC, Power AJ, Walker D. Genetic sex determination, gender identification, and pseudohermaphroditism in the knobbed whelk, *Busycon carica* (Mollusca: Melongenidae). *Proc R Soc Lond B* 2004, **271**: 641~646

6 Segelbacher G, Paxton RJ, Steinbruck G, Trontelj P, Storch I. Characterization of microsatellites in capercaillie *Tetrao urogallus*. *Mol Ecol*, 2000, **9**: 1919~1952

7 Höglund J, Piertney SB, Alatalo RV, Lindell J, Lundberg A, Rintamäki PT. Inbreeding depression and male fitness in black grouse. *Proc R Soc B*, 2002, **269**: 711~715

8 Höglund J, Larsson JK, Jansman HAH, Segelbacher G. Genetic variability in European black grouse (*Tetrao tetrix*). *Conserv Genet*, 2007, **8**: 239~243

9 Larsson JK, Sun YH, Fang Y, Segelbacher G, Höglund J. Microsatellite variation in a Chinese grouse *Bonasa sewerzowi* population: Signs of genetic impoverishment? *Wildl Biol*, 2003, **9**: 261~266

10 Larsson JK, Jansman HAH, Segelbacher G, Höglund J, Koelewijn HP. Genetic impoverishment of the last black grouse (*Tetrao tetrix*) population in the Netherlands: detectable only with a reference from the past. *Mol Ecol*, 2008, **17**: 1897~1904

11 ICGSC. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature*, 2004, **432**: 695~716

12 Sun YH (孙悦华). Distribution, reproduction strategy and population biology of the Chinese grouse (*Bonasa sewerzowi*): [Doctor Degree Dissertation]. Beijing, China: Beijing Normal University (北京: 北京师范大学), 2004

13 Wang J (王杰). Foraging strategy, primary sex ratio and extra-pair paternity of the Chinese grouse (*Tetrastes sewerzowi*): [Doctor Degree Dissertation]. Beijing, China: Institute of Zoology, Chinese Academy of Sciences (北京: 中国科学院动物研究所), 2009

14 Bergmann HH, Klaus S, Muller F, Scherzinger W, Swenson JE, Wiesner J. Die Haselhühner *Bonasa bonasia* und *B.* sewerzowi. In: Die Neue Brehm-Bücherei (Band **77**). Magdeburg, Germany: Westarp Wissenschaften, 1996

15 Wang N, Zhang ZW. The novel primers for sex identification in the brown eared-pheasant and their application to other species. *Mol Ecol Resour*, 2009, **9**: 186~188

16 Kumar S, Tamura K, Nei M. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform*, 2004, **5**: 150~163

17 Rousset F. GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Mol Ecol Res*, 2008, **8**: 103~106